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[Name of Document] Abstract 1

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[Name of Document] CLAIMS

[Claim 1] A method of forming a planar lipid-bilayer membrane for membrane protein analysis, the method comprising the steps of:

(a) filling a microchannel with a buffer solution, the microchannel being disposed under a horizontal partition wall having an aperture;

(b) applying a small amount of a lipid solution as a droplet to the aperture filled with the buffer solution to form a thin layer of the lipid solution in a chamber, the chamber being formed at a position corresponding to the aperture of the partition wall and being provided with a liquid trap on the partition wall inside the chamber; and

(c) applying a buffer solution as a droplet to the chamber from the upper side thereof.

[Claim 2] The method of forming a planar lipid-bilayer membrane for membrane protein analysis according to claim 1, wherein the thickness of the thin layer of the lipid solution is controlled.

[Claim 3] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 1 or 2, wherein the buffer solution contains a liposome (spherical vesicle of a lipid-bilayer membrane) incorporated with an objective membrane protein, and the liposome is fused with the planar lipid-bilayer membrane to incorporate the

membrane protein into the planar lipid-bilayer membrane.

[Claim 4] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 1, wherein a plurality of the chambers are integrally formed.

[Claim 5] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 4, wherein the plurality of the chambers are formed in an array.

[Claim 6] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein liposomes each containing a different protein are each applied to a different chamber, and different kinds of proteins are simultaneously measured.

[Claim 7] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein the reaction/binding of different kinds of reagents or different kinds of proteins in the chambers are measured.

[Claim 8] The method of forming a lipid-bilayer membrane for membrane protein analysis according to claim 4 or 5, wherein the temperature of chamber is independently controlled, liposomes each containing a different protein are each applied to a different chamber, and the proteins different in temperature are simultaneously measured.

[Claim 9] A device for forming a lipid-bilayer membrane for membrane protein analysis, the device comprising:

(a) a substrate;

(b) a microchannel defined by the substrate and a horizontal partition wall;

(c) a chamber provided with an aperture formed in the partition wall and a liquid trap formed at the periphery of the aperture; and

(d) a microinjection device for applying droplets of a lipid solution and a buffer solution to the chamber from the upper side of the chamber.

[Claim 10] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 9, the device further comprising a first thin-film electrode disposed on the substrate at the position corresponding to the chamber and a second thin-film electrode disposed near the liquid trap.

[Claim 11] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 9 or 10, wherein the partition wall has a channel connected to the liquid trap for controlling the thickness of the layer of the lipid solution.

[Claim 12] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 9 or 10, wherein a plurality of the chambers are integrally formed.

[Claim 13] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 12, wherein the plurality of the chambers are formed in an array.

[Claim 14] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, wherein the microinjection device further includes a cover for positioning the microinjection device relative to each chamber.

[Claim 15] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, the device further comprising a means for applying liposomes each containing a different protein to the respective chambers and simultaneously measuring the different kinds of proteins.

[Claim 16] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 12 or 13, the device further comprising a means for independently controlling the temperature of each chamber in an array, applying liposomes each containing a different protein to the respective chamber, and simultaneously measuring the proteins different in temperature.

[Claim 17] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 9, wherein the aperture is provided with a taper so that the diameter of the aperture narrows from the lower side toward the upper side.

[Claim 18] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 9, wherein

the partition wall is formed of a silicon substrate and the aperture is formed by etching the silicon substrate.

[Claim 19] The device for forming a lipid-bilayer membrane for membrane protein analysis according to claim 10, the device further comprising a means for measuring a property of the membrane protein by applying a voltage between the first thin-film electrode and the second thin-film electrode.

[Name of Document] SPECIFICATION

[Title of the Invention] METHOD AND DEVICE FOR FORMING LIPID-BILAYER MEMBRANE FOR MEMBRANE PROTEIN ANALYSIS

[Technical Field]

[0001]

The present invention relates to methods of forming lipid-bilayer membranes for membrane protein analysis and devices therefor. The lipid-bilayer membranes are used in fields such as biotechnology, biochips, membrane protein analysis, drug discovery screening, and biosensors.

[Background Art]

[0002]

As typical methods for producing lipid membranes used in analysis of membrane proteins such as ion channels, painting method and Langmuir-Blodgett method (LB method) are conventionally known. Both methods are of forming a lipid-bilayer membrane in an aperture opened in a Teflon

(registered trademark) sheet in a chamber filled with a buffer solution. The aperture has a size of several-hundred microns. In the painting method, a lipid solution is applied to the aperture with a brush. The LB method utilizes the fact that a monomolecular layer of a lipid molecule is formed on the surface of a solution. In this method, a planar lipid membrane is formed by gradually raising the solution surface level at both sides of a Teflon (registered trademark) sheet in a chamber.

[0003]

FIG. 10 is a schematic diagram showing a method of forming a planar lipid membrane by the LB method.

[0004]

In the drawing, a reference numeral 1 denotes a Teflon (registered trademark) sheet, a reference numeral 2 denotes an aperture formed in the Teflon (registered trademark) sheet 1, a reference numeral 3 denotes a solution on the surface of which a monomolecular layer 4 of lipid is formed, and a reference numeral 5 denotes a buffer solution. A lipid membrane 6 is formed by gradually raising the surface level of the solution 3 at both sides of the Teflon (registered trademark) sheet 1 in a chamber.

Patent Document 1: Japanese Unexamined Patent Application Publication No. 02-35941

Patent Document 2: Japanese Unexamined Patent Application

Publication No. 05-253467

Patent Document 3: Japanese Unexamined Patent Application Publication No. 07-241512

Patent Document 4: Japanese Unexamined Patent Application Publication No. 2002-505007

Patent Document 5: Japanese Unexamined Patent Application Publication No. 2003-511679

Patent Document 6: Japanese Patent Application No. 2003-329667

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Non-Patent Document 2: B. Alberts, et al., "Molecular Biology of the Cell; 4th Ed.", Garland Science, 2002.

Non-Patent Document 3: C. Miller, ed., "Ion Channel Reconstitution", Plenum Press, 1986.

Non-Patent Document 4: T. Ide and T. Yanagida, "An Artificial Lipid Bilayer Formed on an Agarose-Coated Glass for Simultaneous Electrical and Optical Measurement of Single Ion Channels," Biochem. Biophys. Res. Comm., 265, pp. 595-599, 1999.

Non-Patent Document 5: T. Ide, Y. Takeuchi, and T. Yanagida, "Development of an Experimental Apparatus for Simultaneous Observation of Optical and Electrical Signals from Single Ion Channels," Single Molecules, 3(1), pp. 33-42, 2002.

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Non-Patent Document 7: M. Mayer, et al., "Microfabricated Teflon Membranes for Low-Noise Recording of Ion Channels in Planar Lipid Bilayers," Biophys. J., Vol. 85, pp. 2684-2695, 2003.

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Non-Patent Document 9: H. Suzuki, H. Noji, S. Takeuchi, SEIBUTSU BUTSURI (Biophysics), Vol. 43, SUPPLEMENT 1, p. S118, B374, August 2003

[Disclosure of Invention]

[Problems to be Solved]

[0005]

Both methods mentioned above require large chambers of about several centimeters in size. Therefore, the dead volumes are large and microscopic observation cannot be performed. Additionally, in these methods, when a plurality of planar membranes are simultaneously formed in a channel by providing a plurality of apertures, adjacent apertures (planar membranes) are electrically connected to each other through a buffer solution in the channel and the electrophysiological measurement of each membrane is

difficult.

[0006]

Furthermore, basically, only one lipid-bilayer membrane is formed at a time. Therefore, multichannel analysis is impossible. In addition, these methods require an experienced skill and their repeatability is low.

[0007]

The present inventors have already proposed a method of forming an artificial lipid membrane and a device therefor, in which a first and second microchannels are formed and the flow of a lipid solution in the second microchannel is controlled so that a planar lipid-bilayer membrane is formed.

[0008]

In the method, firstly, the first microchannel is filled with a buffer solution (aqueous solution), and then the second microchannel having an aperture is filled with a lipid solution. Then, the lipid solution is discharged by infusing air to the second microchannel. A part of the lipid solution remains at the interface of the buffer solution in the aperture at this step. Then, the buffer solution is introduced into the second microchannel to discharge the air. The air is replaced with the buffer solution and a planar lipid-bilayer membrane is thereby formed in the aperture (the Patent Document 6).

[0009]

However, in this method, the number of the steps for the formation of a planar lipid membrane is large and the process is complicated. In addition, it is difficult to control the thickness of the planar lipid membrane.

[0010]

Recently, it has been required to apply different kinds of reagents or different kinds of proteins to a multi-chamber device and to measure their reaction/binding.

[0011]

Under such circumstances, it is an object of the present invention to provide a method of forming a lipid-bilayer membrane for membrane protein analysis, which is capable of downsizing, simplifying, and multichanneling of a device therefor.

[Means for Solving the Problem]

[0012]

The object of the present invention is achieved by the following aspects:

[1] In a method of forming a planar lipid-bilayer membrane for membrane protein analysis, a microchannel is filled with a buffer solution. The microchannel is disposed under a horizontal partition wall having an aperture. A chamber is formed at a position corresponding to the aperture and is provided with a liquid trap on the partition wall inside the chamber. A small amount of a lipid solution

is applied as a droplet to the aperture filled with the buffer solution to form a thin layer of the lipid solution. A buffer solution is applied as a droplet to the chamber from the upper side of the chamber to thereby form a planar lipid-bilayer membrane.

[0013]

[2] In the method of forming a planar lipid-bilayer membrane for membrane protein analysis according to the aspect [1], the thickness of the thin layer of the lipid solution is controlled.

[0014]

[3] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [1] or [2], the buffer solution contains a liposome (spherical vesicle of a lipid-bilayer membrane) incorporated with an objective membrane protein. The liposome is fused with the planar lipid-bilayer membrane to incorporate the membrane protein into the planar lipid-bilayer membrane.

[0015]

[4] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [1], a plurality of the chambers are integrally formed.

[0016]

[5] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [4],

the plurality of the chambers are formed in an array.

[0017]

[6] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [4] or [5], liposomes each containing a different protein are each applied to a different chamber, and different kinds of proteins are simultaneously measured.

[0018]

[7] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect of [4] or [5], the reaction/binding of different kinds of reagents or different kinds of proteins in each of the chambers are measured.

[0019]

[8] In the method of forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [4] or [5], the temperature of chamber is independently controlled. Liposomes each containing a different protein are each applied to a different chamber, and the proteins different in temperature are measured.

[0020]

[9] A device for forming a lipid-bilayer membrane for membrane protein analysis includes a substrate, a microchannel defined by the substrate and a horizontal partition wall, a chamber provided with an aperture formed

in the partition wall and a liquid trap formed at the periphery of the aperture, and a microinjection device for applying droplets of a lipid solution and a buffer solution to the chamber from the upper side of the chamber.

[0021]

[10] The device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [9] further includes a first thin-film electrode disposed on the substrate at the position corresponding to the chamber and a second thin-film electrode disposed near the liquid trap.

[0022]

[11] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [9] or [10], the partition wall has a channel connected to the liquid trap for controlling the thickness of the layer of the lipid solution.

[0023]

[12] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [9] or [10], a plurality of the chambers are integrally formed.

[0024]

[13] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [12], the plurality of the chambers are formed in an array.

[0025]

[14] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [12] or [13], the microinjection device further includes a cover for positioning the microinjection device relative to each chamber.

[0026]

[15] The device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [12] or [13] further includes a means for applying liposomes each containing a different protein to the respective chambers and simultaneously measuring the different kinds of proteins.

[0027]

[16] The device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [12] or [13] further includes a means for independently controlling the temperature of each chamber in an array, applying liposomes each containing a different protein to the respective chambers, and simultaneously measuring the proteins different in temperature.

[0028]

[17] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [9], the aperture is provided with a taper so that the diameter of the aperture narrows from the lower side toward the upper side.

[0029]

[18] In the device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [9], the partition wall is formed of a silicon substrate and the aperture is formed by etching the silicon substrate.

[0030]

[19] The device for forming a lipid-bilayer membrane for membrane protein analysis according to the aspect [10] further includes a means for measuring a property of the membrane protein by applying a voltage between the first thin-film electrode and the second thin-film electrode.

[Effect of the Invention]

[0031]

According to the present invention, the following advantageous effects are achieved:

[0032]

(1) The device for forming a lipid-bilayer membrane for membrane protein analysis includes a substrate, a microchannel defined by the substrate and a horizontal partition wall, a chamber provided with an aperture formed in the partition wall and a liquid trap formed at the periphery of the aperture. The amount of a lipid solution can be precisely controlled and injected to the chamber from the upper side of the chamber with a microinjection device (microinjector). Lipid-bilayer membranes with high

repeatability can be readily formed (reconstituted).

[0033]

(2) Since the apertures and chambers disposed in an array are independent to each other as a measurement system, many kinds of measurements can be simultaneously conducted. Therefore, fast membrane protein analysis can be achieved.

[0034]

(3) Since the measurement system and the channel for injecting a reagent are fabricated in a microscale (less than 1 mm), the dead volume is considerably reduced to significantly decrease the amounts of the reagent and the sample of necessary.

[0035]

(4) Since the size of the measurement system is very small, the measurement is not easily affected by external electric noise. Thus, the electrical measurement can be further precisely performed.

[Best Mode for Carrying Out the Invention]

[0036]

According to the present invention, a planar lipid-bilayer membrane is formed: a microchannel is filled with a buffer solution, the microchannel being disposed under a horizontal partition wall having an aperture; a chamber being formed at a position corresponding to the aperture and provided with a liquid trap on the partition wall inside the

chamber; a small amount of a lipid solution is applied as a droplet to the aperture filled with the buffer solution to form a thin layer of the lipid solution in a channel,; and a buffer solution is applied as a droplet to the chamber from the upper side of the chamber to thereby form the planar lipid-bilayer membrane. Consequently, the amount of the lipid solution to be introduced to the chamber can be precisely controlled, and the lipid-bilayer membrane can be readily formed (reconstituted) with a high repeatability.

[0037]

The present invention will now be described in detail with reference to the embodiments.

[First Embodiment]

[0038]

FIG. 1 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a first embodiment of the present invention. FIG. 2 is a schematic diagram showing a lipid solution.

[0039]

In FIG. 1, a reference numeral 11 denotes a glass substrate, a reference numeral 12 denotes a lower microchannel, a reference numeral 13 denotes a partition wall, a reference numeral 14 denotes an aperture (opening) provided to the partition wall 13, a reference numeral 15 denotes a liquid trap provided on the partition wall 13, a

reference numeral 17 denotes a chamber defined by a well 16, a reference numeral 18 denotes a buffer solution which fills the lower microchannel 12 and the aperture (opening) 14, a reference numeral 19 denotes a microinjection device (microinjector), a reference numeral 20 denotes a lipid solution applied as a droplet from the microinjection device 19, a reference numeral 21 denotes a planar lipid layer, a reference numeral 22 denotes a microinjection device (microinjector or pipette) for applying a buffer solution as a droplet, a reference numeral 23 denotes a buffer solution applied as a droplet from the microinjection device 22, and a reference numeral 24 denotes a planar lipid-bilayer membrane.

[0040]

In the device for forming (reconstituting) a planar lipid-bilayer membrane, as described above, the lower microchannel 12 and the chamber 17 are separated from each other by the partition wall 13 having the aperture (opening) 14.

[0041]

Firstly, as shown in FIG. 1(a), the lower microchannel 12 and the aperture 14 are filled with the buffer solution 18 (KCl or aqueous solution). At this stage, the interface of the buffer solution 18 stops at the aperture (opening) 14 due to the surface tension. Here, the aperture (opening) 14

is provided with a taper 13A so that the diameter of the aperture 14 narrows from the lower side toward the upper side. Thus, the interface of the buffer solution 18 readily stops at the aperture (opening) 14.

[0042]

Then, as shown in FIG. 1(b), the lipid solution 20 is injected to the aperture (opening) 14 by using the microinjection device 19. At this stage, the excess of the lipid solution 20 flows into the liquid trap 15 provided at the periphery of the aperture (opening) 14. Accordingly, the layer (planar lipid layer 21) of the remaining lipid solution 20 at the interface of the buffer solution 18 can be sufficiently thinned (submicrometer order).

[0043]

Lastly, as shown in FIG. 1(c), the buffer solution 23 is applied as a droplet to the chamber 17 by using the microinjection device 22, and thereby a planar lipid-bilayer membrane (thickness: about 10 nm) 24 is spontaneously formed.

[0044]

As described above, (1) the lower microchannel 12 and the aperture 14 are filled with the buffer solution 18, (2) a small amount of the lipid solution 20 is applied as a droplet, and (3) the buffer solution 23 is applied to the chamber 17 as a droplet. As a result, a layer (planer lipid layer 21) of the lipid solution 20 is spontaneously

assembled to a planar lipid-bilayer membrane 24.

[0045]

Here, as shown in FIG. 2(a), the lipid solution 20 includes a component (phospholipids) having a hydrophilic group 20A and a hydrophobic group 20B. By thinning the lipid solution layer, as shown in FIG. 2(b), the hydrophobic group 20B is arranged to face inside, and engaged and bound to each other to form the planar lipid-bilayer membrane 24.

[0046]

For forming the bilayer, the layer of the lipid solution must be thinned as much as possible (nm order). Then, a means for controlling the thickness of the layer by communication with the lipid trap 15 may be provided, as described below.

[Second Embodiment]

[0047]

FIG. 3 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a second embodiment of the present invention.

[0048]

In a second embodiment, in addition to the components in the first embodiment, a first thin-film electrode 25 is provided on the glass substrate 11 of the microchannel 12 and a second thin-film electrode 26 is provided on the partition wall 13 within the chamber 17 defined by the well

16. Since the microelectrodes 25 and 26 are independently provided in the chamber 17 defined by the well 16, the membrane potential and current can be measured.

[0049]

In order to incorporate a membrane protein to be analyzed into the planar lipid-bilayer membrane 24 formed in the microchannel, a spherical vesicle (liposome) of the same lipid bilayer is used.

[0050]

As shown in FIG. 4, a liposome 31 containing a membrane protein 32 is prepared. Droplets of the liposome is mixed in the buffer solution 23, and introduced to the planar lipid-bilayer membrane 24 as a droplet. The liposome 31 and the planar lipid-bilayer membrane 24 are spontaneously fused to each other by the contact of the liposome 31 with the planar lipid-bilayer membrane 24, and the membrane protein 32 is incorporated into the planar lipid-bilayer membrane 24. The present inventors have succeeded, as a test case, to insert Alamethicin into a planar lipid-bilayer membrane by fusing a liposome containing Alamethicin to the planar lipid-bilayer membrane formed by a known planar lipid-bilayer method. Alamethicin is a peptide that stochastically forms ion channels by oligomerization, changing transiently between its open and close states. The membrane current was measured with the addition of the

buffer solution containing Alamethicin to confirm the fusion of the membrane protein (peptide) to the bilayer.

[Third Embodiment]

[0051]

FIG. 5 is a schematic diagram of a device for forming a planar lipid-bilayer membrane according to a third embodiment of the present invention.

[0052]

In this embodiment, the device is provided with a channel 12A connecting to the liquid trap 15. Therefore, it is possible to control the thickness of the layer of the lipid solution 20 remaining on the interface of the buffer solution 18. In other words, when a thick lipid solution layer 21 is formed by the lipid solution 20 remaining on the interface of the buffer solution 18, the thickness of the planar lipid layer 21 can be decreased by sucking the excess of the lipid solution 20 through the channel 12A connecting to the liquid trap 15. Reversely, when a thin planar lipid layer 21 is formed by the lipid solution 20 remaining on the interface of the buffer solution 18, the thickness of the planar lipid layer 21 can be increased by pushing the lipid solution 20 back through the channel 12A connecting to the liquid trap 15.

[Fourth Embodiment]

[0053]

FIG. 6 is schematic diagrams of a device for forming a planar lipid-bilayer membrane according to a fourth embodiment of the present invention. FIG. 6(a) is a perspective view of the top face of chambers in an array. FIG. 6(b) is a cross-sectional view of a well array chip.

[0054]

In the drawings, a reference numeral 41 denotes a glass substrate, a reference numeral 42 denotes a lower microchannel, a reference numeral 43 denotes a partition wall formed of silicon, a reference numeral 44 denotes an aperture formed by etching the partition wall 43, a reference numeral 45 denotes a liquid trap formed at the periphery of the aperture 44, a reference numeral 47 denotes a chamber defined by a well 46, a reference numeral 48 denotes a buffer solution which fills the lower microchannel 42 and the aperture 44, a reference numeral 49 denotes a planar lipid-bilayer membrane, a reference numeral 50 denotes a buffer solution applied as a droplet on the planar lipid-bilayer membrane 49, a reference numeral 51 denotes a first thin-film electrode disposed on the glass substrate 41 at the position under the aperture 44, a reference numeral 52 denotes a second thin-film electrode disposed at the periphery of the liquid trap 45, and a reference numeral 53 denotes a power source with ammeter disposed between the first thin-film electrode 51 and the second thin-film

electrode 52.

[0055]

As described above, in this embodiment, the chambers 47 are each defined by a well 46 and disposed in an array.

[0056]

Therefore, a plurality of kinds of membrane proteins can be simultaneously measured by applying liposomes each containing a different membrane protein to the respective chambers 47. The different membrane proteins are each incorporated into the planar lipid-bilayer membranes formed in an array according to the present invention with a microinjection device for a reagent. Then, the membrane proteins are simultaneously measured with a multichannel system. For example, membrane proteins A and B are separately incorporated into different planar lipid-bilayer membranes. When a reagent which suppresses or activates either of these membrane proteins is applied to chambers through the channel, electric signals of the membrane proteins A and B are different from that of each other. In addition, another signal can be obtained by applying a reagent having another effect. Thus, a plurality of measurements can be simultaneously performed with a high sensitivity to analyze how the membrane proteins react to which reagents.

[0057]

A measuring system (not shown) according to the present invention includes a planar lipid-membrane chip, an injection device for injecting a membrane protein (liposome), a syringe pump for injecting a reagent, an amplifier (patch amplifier) for amplifying small membrane current/voltage, and a computer for result analysis. Firstly, planar lipid-bilayer membranes are formed in an array according to the present invention. Liposomes each containing a different objective membrane protein are applied to the planar lipid-bilayer membranes with the microinjection device. The membrane currents/voltages when various reagents are applied to each membrane protein through the lower microchannel are measured by using the microelectrodes, and signals amplified by the amplifier are incorporated into the computer. The computer analyzes the output signals. Thus, identification and functional analysis of each membrane protein can be performed.

[0058]

In addition, the temperature of each chamber in an array may be independently controlled. Liposomes each containing a different membrane protein are applied to the planar lipid-bilayer membranes. Thus, the proteins different in temperature may be simultaneously measured. In such a case, a heating device (not shown) is provided to each chamber.

[0059]

FIG. 7 is cross-sectional views of a well array chip of the devices for forming planar lipid-bilayer membranes in a fabrication process thereof according to the fourth embodiment of the present invention.

[0060]

(1) Firstly, as shown in FIG. 7(a), oxide films 62 are formed on the top and bottom faces of a silicon substrate 61.

[0061]

(2) Then, as shown in FIG. 7(b), one of the oxide films 62 is patterned and tiny holes (50 to 100 μm in width and 200 μm in depth) 63 are formed by reactive ion etching.

[0062]

(3) Then, as shown in FIG. 7(c), a lower microchannel 64 and an aperture 65 are etched using tetramethylammonium hydroxide (TMAH).

[0063]

(4) Then, as shown in FIG. 7(d), a liquid trap 66 is formed at the periphery of the aperture 65 by etching the oxide film 62 and the silicon substrate 61.

[0064]

(5) Then, as shown in FIG. 7(e), the entire chip is coated with Parylene C 67 for the electrical insulation.

[0065]

(6) Finally, as shown in FIG. 7(f), a lower electrode

68 and a glass substrate 69 are bonded to the bottom side. On the top side, an upper electrode (Au) 70 is patterned and a well 71 is formed of a resist (SU8: product name) of 40 μm in thickness.

[0066]

FIG. 8 is an enlarged plan view of a part of an array of the devices for forming planar lipid-bilayer membranes according to the fourth embodiment of the present invention. FIG. 8(a) shows an array chip, and FIG. 8(b) shows an enlarged view of the chip.

[0067]

In these drawings, apertures 65 are each surrounded by a liquid trap 66 formed in a shape of a square trench. At the periphery of each liquid trap 66 formed in a shape of a square trench, an upper electrode 70 is disposed. SU8 wells 71 are formed so as to define each chamber.

[0068]

Here, in the drawings, the size of the aperture 65 formed at the center is 200 μm, the size of the well 71 is 900 μm. The size and depth of the liquid trap 66 are 500 μm and 40 μm, respectively. The capacity of the liquid trap 66 is 8 nL (8 nanoliters). The upper electrodes 79 are separated for each chamber and the lower electrode 68 is common to all chambers.

[Fifth Embodiment]

[0069]

FIG. 9 is a perspective view of a microinjection device of an array of the devices for forming planar lipid-bilayer membranes according to a fifth embodiment of the present invention.

[0070]

In this drawing, reference numerals 81 to 89 denote nozzles of the microinjection device. Each of the nozzles corresponds to the respective chambers of a well array chip 92. A reference numeral 90 denotes a cover integrated with the nozzles. The cover is provided for positioning the nozzles 81 to 89 of the microinjection device relative to each chamber of the well array chip 92. A reference numeral 91 denotes an engaging member for engaging the cover with the well array chip 92 for the positioning.

[0071]

The drying of the planar lipid-bilayer membrane disadvantageously affects the measurement. By immediately installing the cover 90 to the well array chip 92 after the production of the planar lipid-bilayer membranes having liposomes containing membrane proteins, the drying of the planar lipid-bilayer membranes in an array can be reduced.

[0072]

During the measurement, the drying of buffer solutions in the chambers can be avoided by optionally applying a

buffer solution as a droplet to each chamber through the respective nozzles 81 to 89 of the microinjection device.

[0073]

The present invention is not limited to the above-mentioned embodiments. Various modifications based on the concept of the present invention are possible and are within the scope of the present invention.

[Industrial Applicability]

[0074]

The present invention is suitable for biotechnology, biochips, membrane protein analysis, drug discovery screening, and biosensors, and can be applied to an ultrasensitive membrane-protein analysis device, an ultrasensitive multichannel drug discovery screening device, and an ultrasensitive ion sensor.

[Brief Description of the Drawings]

[0075]

[FIG. 1] A schematic diagram of a device for forming a planar lipid membrane according to a first embodiment of the present invention.

[FIG. 2] A schematic diagram of a lipid solution according to the present invention.

[FIG. 3] A schematic diagram of a device for forming a planar lipid-bilayer membrane according to a second embodiment of the present invention.

[FIG. 4] A diagram showing the incorporation of a membrane protein into a planar lipid-bilayer membrane by using a liposome according to the present invention.

[FIG. 5] A schematic diagram of a device for forming a planar lipid-bilayer membrane according to a third embodiment of the present invention.

[FIG. 6] Schematic diagrams of a device for forming a planar lipid-bilayer membrane according to a fourth embodiment of the present invention.

[FIG. 7] Cross-sectional views of a well array chip of the devices for forming planar lipid-bilayer membranes in a fabrication process thereof according to the fourth embodiment of the present invention.

[FIG. 8] Enlarged plan views of a part of an array of the devices for forming planar lipid-bilayer membranes according to the fourth embodiment of the present invention.

[FIG. 9] A perspective view of a microinjection device of an array of the devices for forming planar lipid-bilayer membranes according to a fifth embodiment of the present invention.

[FIG. 10] A schematic diagram showing a method of forming a planar lipid membrane by the LB method.

[Description of Symbols]

[0076]

11, 41, 69: glass substrate

12, 42, 64: lower microchannel

12A: channel

13, 43: partition wall

13A: taper

14, 44, 65: aperture (opening)

15, 45, 66: liquid trap

16, 46, 71: well

17, 47: chamber

18, 48: buffer solution which fills microchannel and aperture (opening)

19: microinjection device (microinjector)

20: lipid solution applied as droplet from microinjection device

20A: hydrophilic group

20B: hydrophobic group

21: planar lipid layer

22: microinjection device for applying buffer solution

23, 50: buffer solution applied as droplet from microinjection device

24, 49: planar lipid-bilayer membrane

25, 51: first thin-film electrode

26, 52: second thin-film electrode

31: liposome

32: membrane protein

53: power source with ammeter

61: silicon substrate

62: oxide film

63: tiny hole

67: Parelyne C

68: lower electrode

70: upper electrode (gold)

81-89: nozzles of microinjection corresponding to each chamber

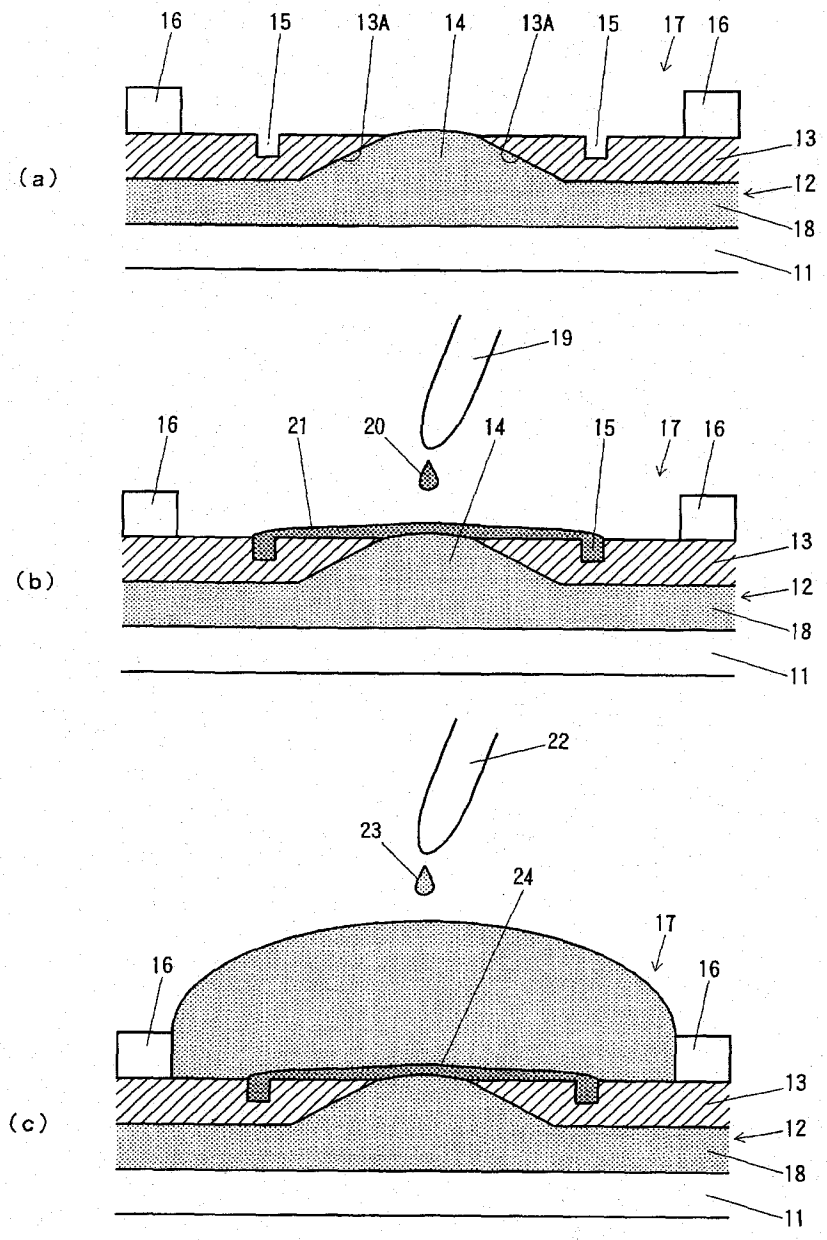
90: cover

91: engaging member

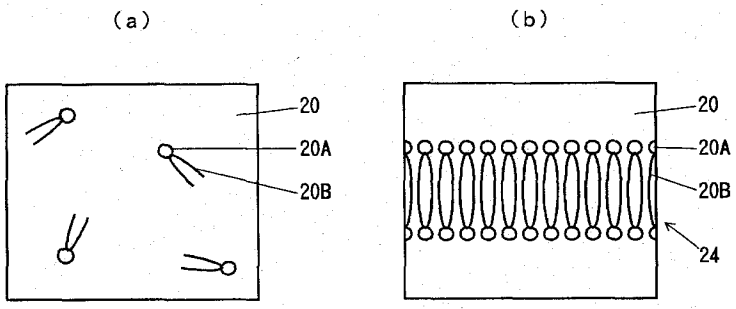
92: well array chip

[Name of Document] DRAWINGS

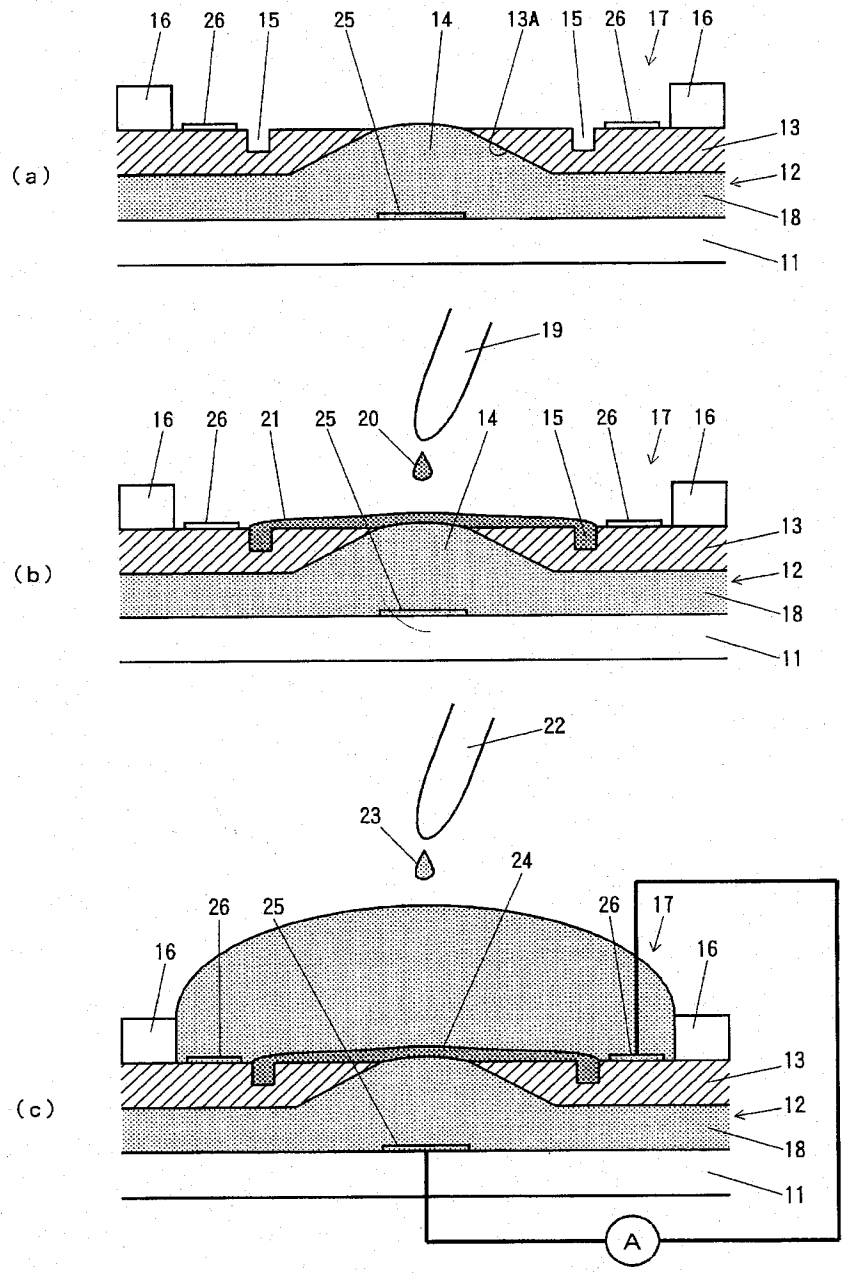
[FIG. 1]



[FIG. 2]



[FIG. 3]


(a)
16
26
15
25
14
13A
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26
17
16
13
12
18
11
(b)
19
16
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21
25
20
14
15
26
17
16
13
12
18
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(c)
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16
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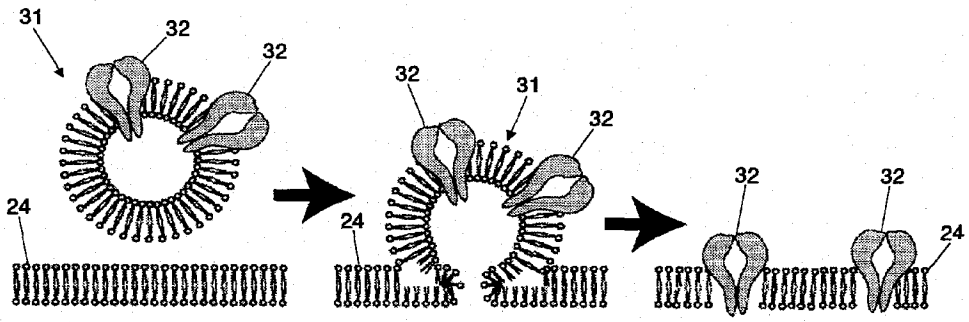
11
A

[FIG. 4]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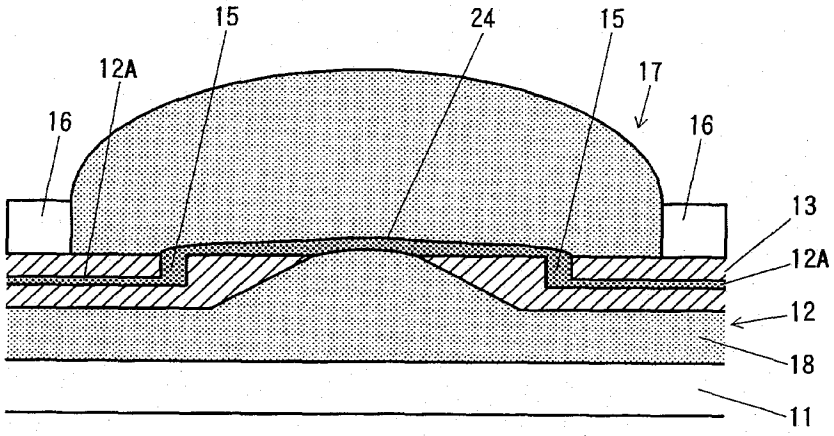
24
FUSION

[FIG. 5]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24
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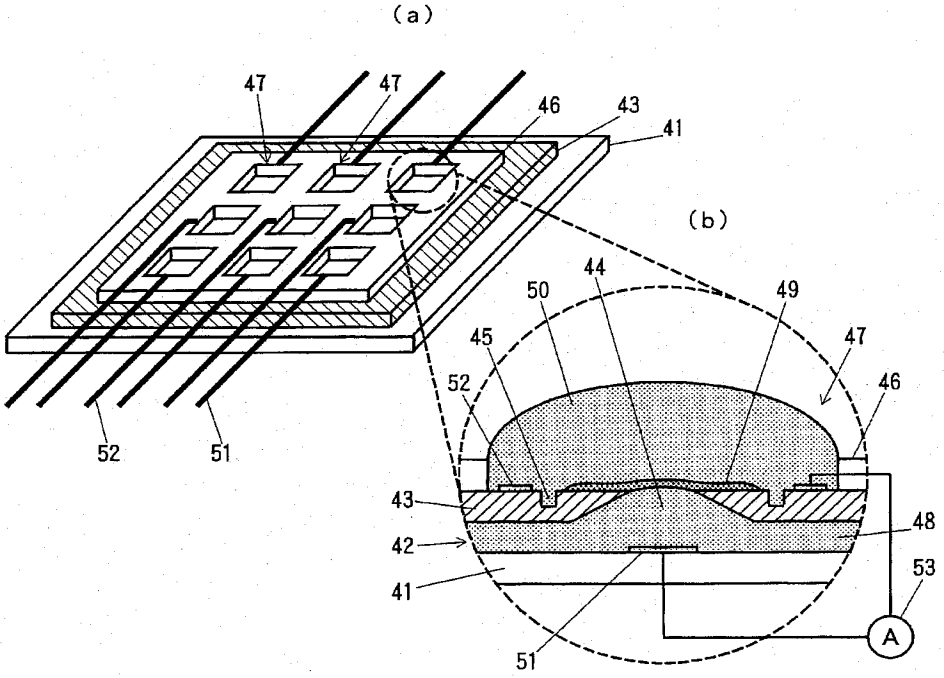
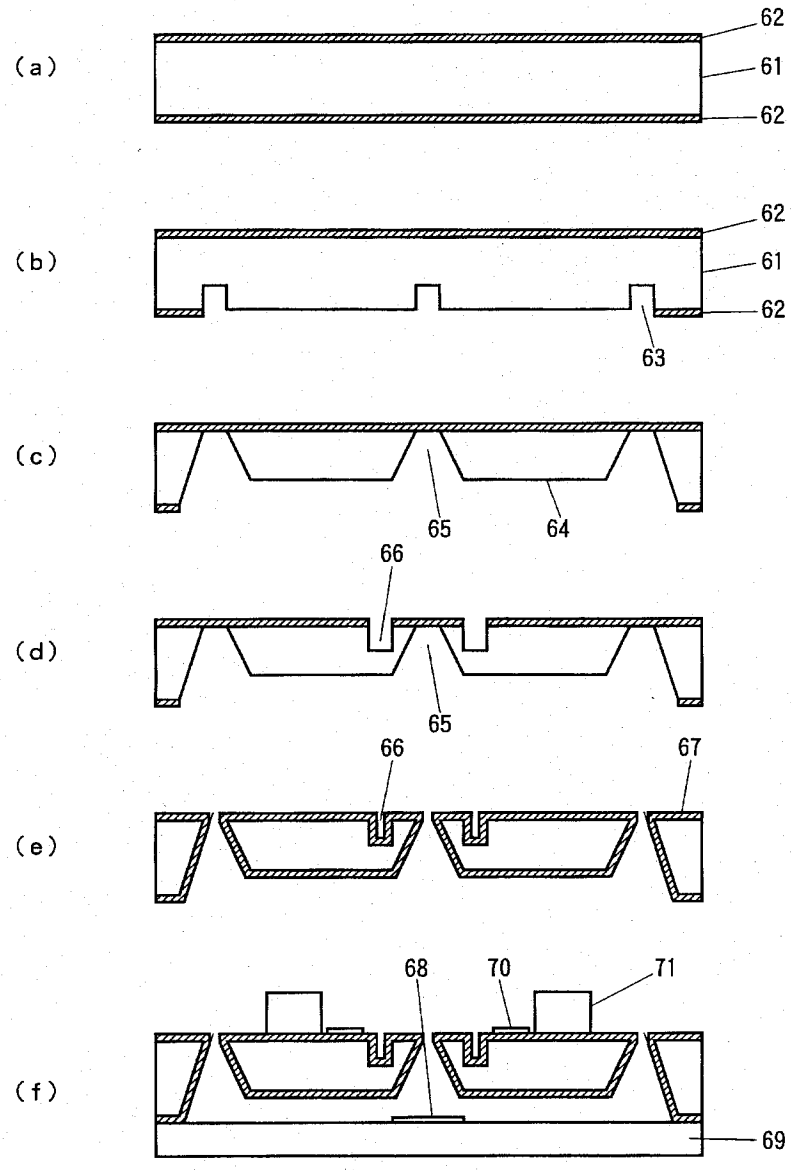
12A
17
16
16
13
12A
12
18
11

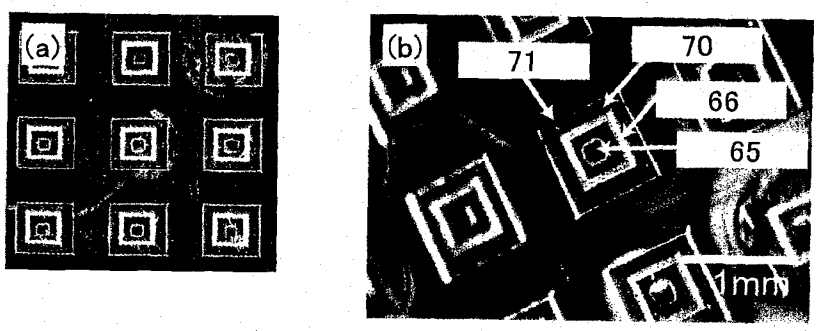
[FIG. 6]



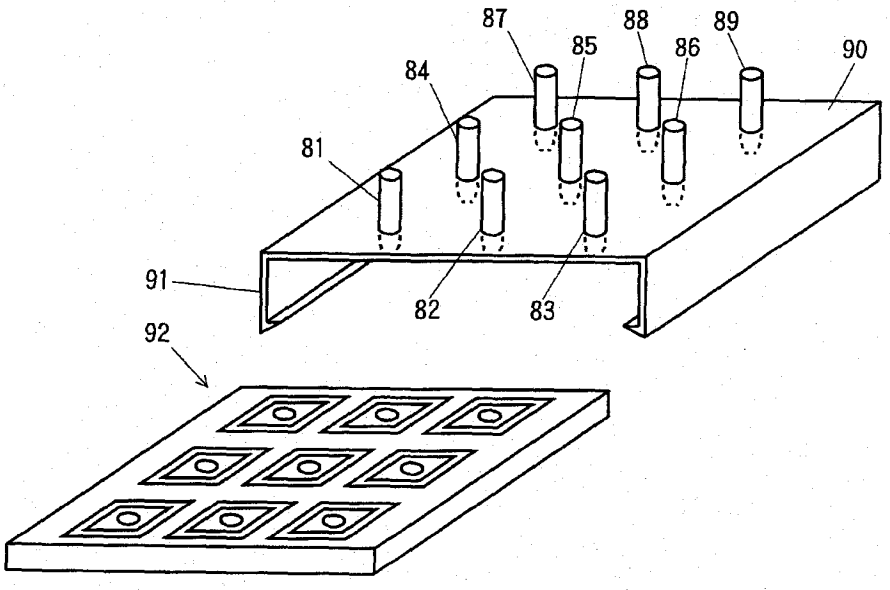
[FIG. 7]



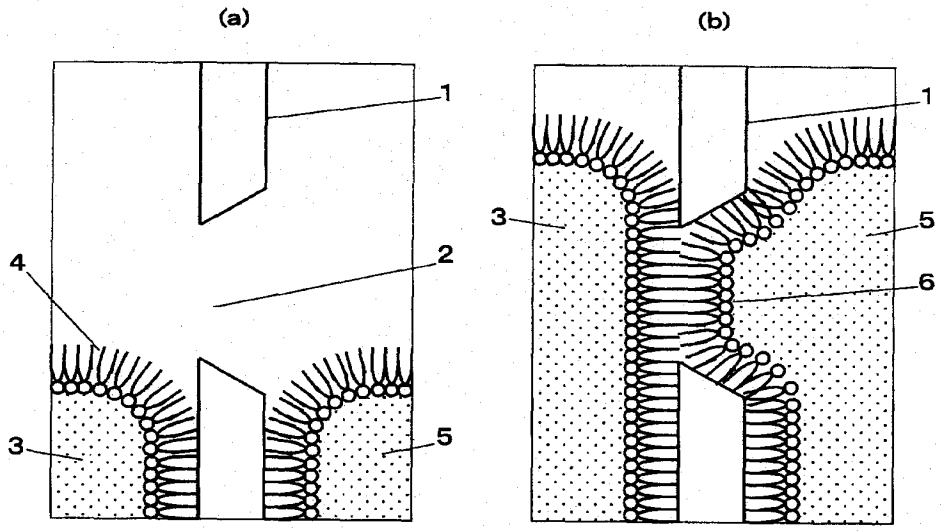
[FIG. 8]



[FIG. 9]



[FIG. 10]



[Name of Document] ABSTRACT

[Abstract]

[Object]

To provide a method of forming a planar lipid-bilayer membrane for membrane protein analysis, by which downsizing, simplifying, and multichanneling of a device therefor are achieved.

[Solving Means]

A planar lipid-bilayer membrane 24 is formed by filling a microchannel 12 with a buffer solution 18, the microchannel 12 disposed under a horizontal partition wall 13 having an aperture 14; applying a small amount of a lipid solution 20 as a droplet on the aperture 14 filled with the buffer solution 18 to thereby form a thin layer 21 of the lipid solution in a chamber, the chamber 17 being formed at a position corresponding to the aperture 14 and provided with a liquid trap 15 inside the chamber; and applying a buffer solution 23 as a droplet to the chamber 17 from the upper side of the chamber.

[Representative Drawing] FIG. 1